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On the architecture and function of cuttlefish bone

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The internal shell of the cuttlefish, which acts as a rigid buoyancy tank, is structured to combine high compressive strength – since it must withstand the external hydrostatic pressure – with minimum weight. The micro-architecture of cuttlebone has been examined by electron microscopy and the relevance of the structure to the mechanical duties required of the shell *in vivo* are briefly discussed. The inorganic calcareous structure is associated with an organic component which may act as a template for mineralization.

1. Introduction

The familiar internal shell or bone of the cuttlefish (*Sepia Officinalis L.*) appears to function both as a skeletal structure and as a rigid buoyancy tank, enabling the cuttlefish to become more or less dense than sea water. Since an animal fractionally denser than sea water can only preserve constant depth by the expenditure of significant energy, the variable buoyancy tank role of cuttlebone confers a considerable advantage to the cuttlefish which can maintain a fixed position in water with little effort. The manner in which the cuttlebone functions as a buoyancy tank has been described by Denton and Gilpin-Brown [1] although there appears to have been few, if any, attempts to describe the microscopic architecture of cuttlebone since the work of Appellöf in 1893 [2] and no serious attempt to relate this architecture to the mechanical requirements imposed on the structure by its role as buoyancy organ.

The cuttlebone (which accounts for about 9% of the animal's volume) is a hollow structure, divided by lamellae, containing both liquid and gas and the cuttlefish changes its density by varying the quantity of liquid within the porous structure of the bone. What is remarkable is that the gas (largely nitrogen) is at a pressure of about 0.8 atm (0.08 MPa), the extraction of liquid from the chamber to increase buoyancy being an active "pump" process [1], and that at a depth of 70 m the pressure difference between interior and exterior of the bone would be about 7 atm (0.7 MPa).

The cuttlebone must then be structurally adapted for rigidity as a skeletal structure and as a strong but light tank able to withstand significant external pressure. The following study shows how well the microscopic architecture is adapted to these duties.

2. Experimental details

The disposition of the bone within the cuttlefish is shown in Fig. 1. For the present examination, thoroughly dried bone specimens were used. Fig. 2 shows schematically a transverse section through the bone. It consists of two regions: a thick external wall or dorsal shield and an internal lamellar matrix – the lamellae being separated by numerous pillars. These two regions are described separately below.

2.1. Microstructure of the cuttlebone

2.1.1. Lamellar matrix

Fig. 3 is a scanning electron micrograph of the internal cuttlebone matrix. The parallel sheets (or lamellae) of calcium carbonate form chambers which are sealed from each other, but within any individual chamber gas or liquid can move freely. The spacing of the lamellae varies in different areas of the cuttlebone but is usually between 200 and 600 μm. The "ceiling" of each chamber is supported by numerous pillars as is shown in

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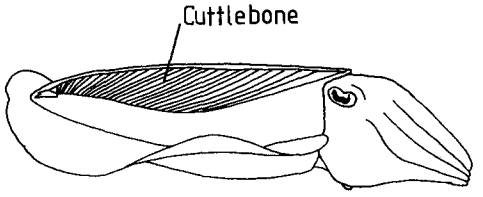

Figure 1 Schematic diagram to show the position of the cuttlebone.

more detail in Fig. 4. The pillars have a sigmoidal cross-section as illustrated in Fig. 5, which is a scanning electron micrograph taken of a section cut parallel to the lamellae. The corrugated appearance of the columns, which is shown more clearly in Fig. 6, seems to be due to growth steps along the length of the column.

The calcareous material of cuttlebone was shown by X-ray diffraction to be aragonite. This crystalline form is presumably stabilized by the presence of strontium, as found in the analysis of Hewitt [3]. The density of the dry internal lamellar matrix was measured as $190 \pm 20\,kg\,m^{-3}$ and, given that the density of aragonite is $2940\,kg\,m^{-3}$, the percentage porosity (neglecting the organic component) is 93%.

2.1.2. The organic component

The shells of all molluscs consist of calcium carbonate associated with an organic component which is probably involved in the shell-forming process (see discussion). Cephalopod shell contains a relatively high percentage of organic material by comparison with other mollusc shells [4]. The organic component was isolated from the cuttlebone lamellar matrix by agitating the shell in 10% HCl to dissolve away the calcium carbonate. Complete decalcification took several days. When the extracted organic phase was dried and weighed, it was found that the cuttlebone lamellar matrix contained between 3 and 4.5% by weight of organic material.

In the internal matrix of the cuttlebone each individual lamella and column appears to be coated in organic material. This is illustrated in Fig. 7, which shows that when the aragonite in

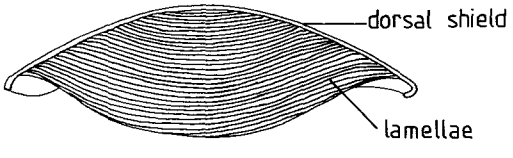

Figure 2 Transverse section through the cuttlebone.

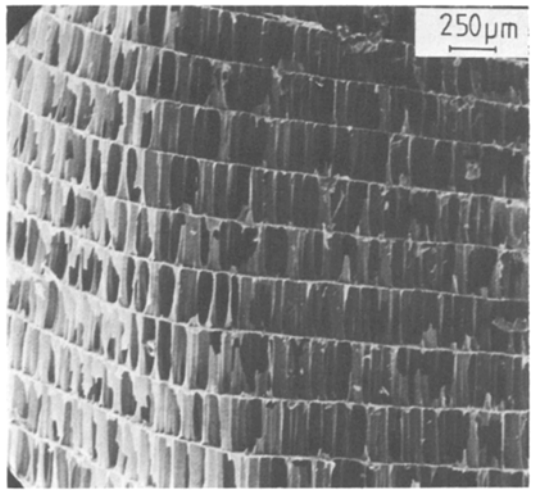

Figure 3 Scanning electron micrograph of lamellar matrix.

cuttlebone has been dissolved away in concentrated HCl there remains an integral, although somewhat collapsed, structure of purely organic content. To investigate further the relationship between the organic and inorganic phases in cuttlebone, a sample was prepared in which the calcium carbonate was only partially dissolved away. Fig. 8 is a scanning electron micrograph of this sample. Retention of part of the inorganic material results in maintenance of the rigidity of the structure. At higher magnification, as in Fig. 9, the relative dispositions of organic and inorganic phases are clearly seen. The inorganic material in the columns is the rigid component with a corrugated appearance and the organic material appears

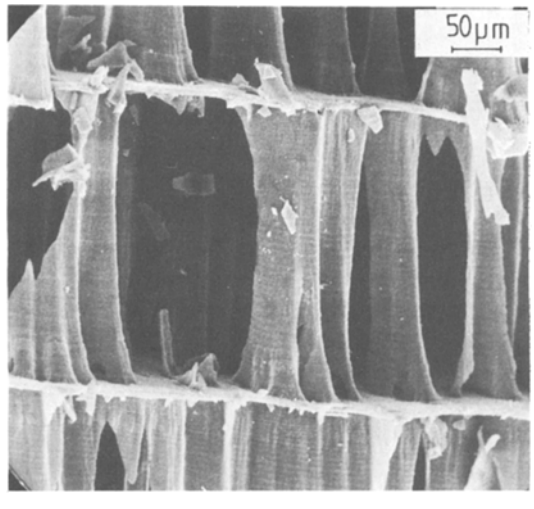

Figure 4 Scanning electron micrograph showing pillars supporting lamellae.

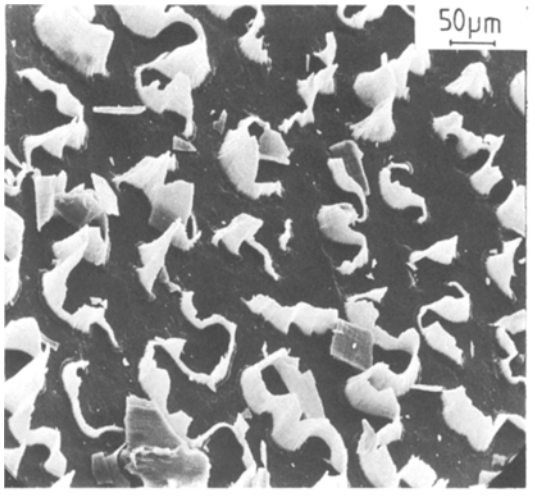

Figure 5 Micrograph illustrating S-shaped cross-section of columns.

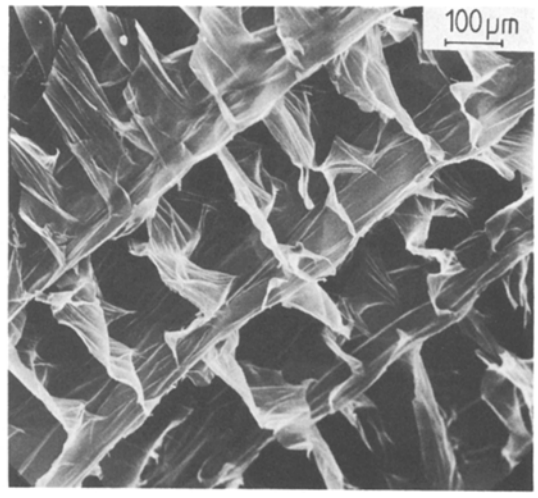

Figure 7 Organic component of structure with aragonite removed by dissolution.

as a thin film covering both sides of the column. Hence each column is enveloped in a membrane of organic material.

The organic component from both the dorsal shield and the internal structure was isolated by repeated extraction of calcium carbonate by dilute HCl. In both samples, a dark blue colouration on treatment with Folin indicated the presence of protein and both materials gave an infrared spectrum [5] identical to that found for crab chitin. Thus the organic component of both dorsal shield and internal structure appears to be a protein–chitin complex, the internal structure containing 3 to 4.5% by weight of this material and the dorsal shield approximately tenfold this amount.

The nature of the organic constituent in mollusc shells has intrigued biochemists for some time. Chitin is one of the most widely distributed of the organic molecules found in skeletal and cuticular structures and is known to be present in the shells of mollusca [6]. It is isotactic poly-N-acetyl-D-glucosamine linked by β-(1-4)-glycosidic bonds. The disaccharide repeating unit is chitobiose. Chitin however does not exist as such in skeletal and cuticular structures but is present in chitin–protein complexes, i.e. glycoproteins or muco-proteins. It therefore rarely constitutes more than

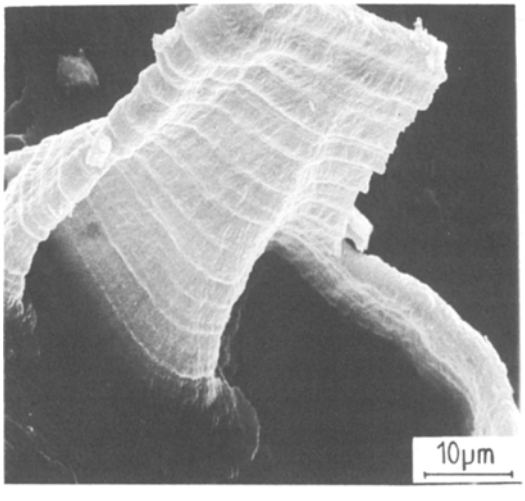

Figure 6 Micrograph showing growth steps along the length of a pillar.

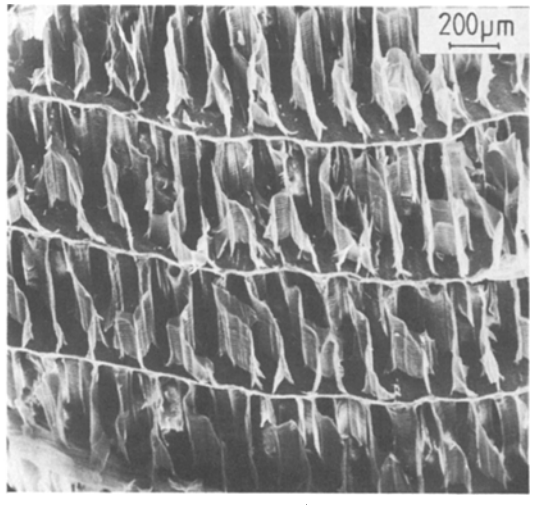

Figure 8 Scanning electron micrograph of lamellar matrix with calcium carbonate partially removed by dissolution.

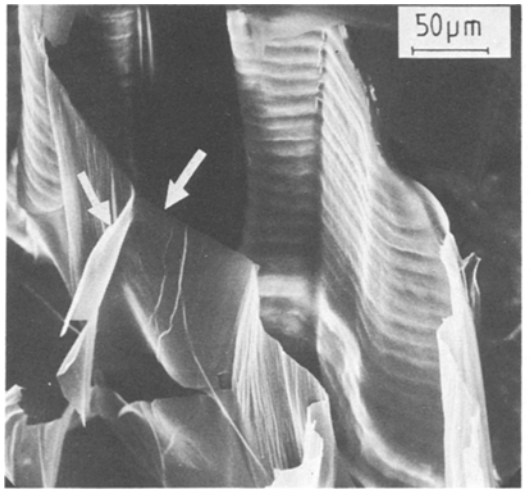

Figure 9 Micrograph showing thin films of organic material (arrowed) between which was the inorganic material in the original structure.

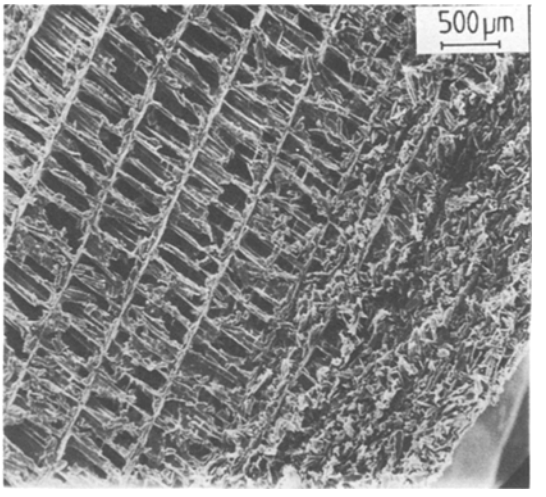

Figure 10 Scanning electron micrograph showing the localized failure of crushed cuttlebone.

50% of the total organic matter in chitinous structures. With reference to cuttlefish, the chitin extracted from both the dorsal and lamellar regions of the bone has been identified as the crystallographic β-type [5, 7]. The chitin is covalently linked to protein and the amino acid composition of the protein components from cuttlefish shell have been identified [8, 4]. The protein in glycoprotein complexes is sclerotized, thus conferring on the structure hardness, rigidity and resistance to enzymic hydrolysis. Besides the stable glycoprotein, most chitinous structures also contain free protein that can be easily extracted by water [6].

2.1.3. Dorsal shield

The dorsal shield (see Fig. 2) is a thick, tough cover which overlays the lamellar matrix and seals off the separate chambers. It consists of three layers: a rigid outer calcified zone about 1 mm thick, a transparent middle zone about 0.3 mm thick composed of tough, fibrous layers of sclerotized chitin, and a thin, inner calcified zone. This layered structure is non-porous and contains 30 to 40% by weight of organic material.

2.2. Mechanical properties

2.2.1. Crushing strength

The crushing strengths of rectangular blocks cut from the cuttlebone lamellar matrix were measured. The load was applied either parallel to or perpendicular to the lamellae. In both cases the samples failed by localized compaction: progressive crushing of layers of the sample occurred from one side, the rest of the sample remaining relatively undeformed. The "local" nature of the crushing process is illustrated in the scanning electron micrograph shown in Fig. 10. In this sample the load was applied perpendicular to the lamellae. The layers on the right-hand side of the sample have been crushed, whereas those on the left remain undeformed. No significant difference in crushing strength was obtained when the load was applied parallel as opposed to perpendicular to the lamellae. The mean crushing strength (from ten samples) was 1.1 ± 0.4 MPa. Part of a typical load against compression plot for a sample crushed with the force applied perpendicular to the lamellae is shown in Fig. 11. The load is maintained while successive layers are crushed.

2.2.2. Flexural strength

The flexural strength of the cuttlebone lamellar matrix, as measured on small beams in a three-point bend test, was found to be 1.8 ± 0.2 MPa. That of the dorsal shield was two orders of magnitude higher: it was found to be 170 MPa.

3. Discussion

Two aspects of this study may intrigue the materials scientist or engineer: firstly, how well the macroscopic and microscopic structure of the cuttlebone is adapted to the duty required of that organ, and secondly, the mechanism by which such a remarkable and complex a structure is formed.

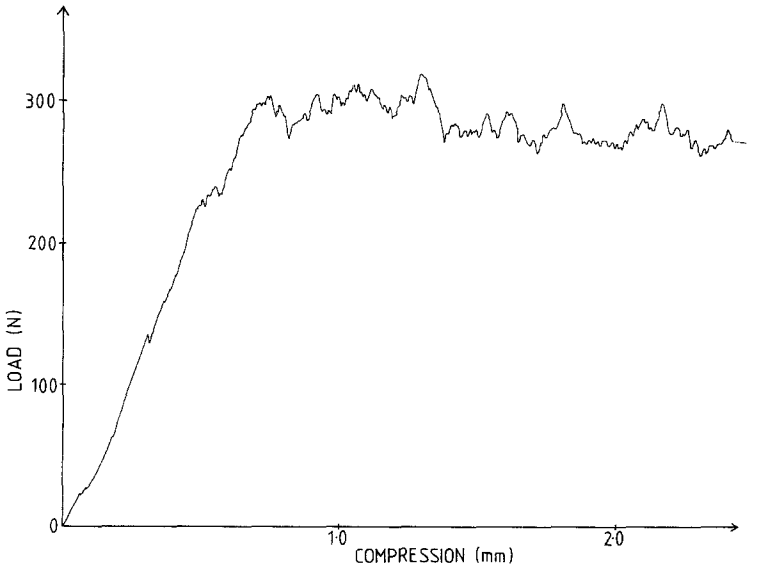

Figure 11 Load against compression plot for a sample of cuttlebone crushed with the force applied perpendicular to the lamellae.

3.1. Architecture and function

The function of the cuttlebone is to act as a rigid buoyancy tank, enabling the animal to maintain a fixed position at a given depth with little effort as it awaits passing prey. The internal gas pressure is maintained at less than 0.1 MPa and the shell must resist the external hydrostatic pressure encountered by the cuttlefish. This ability must not be accompanied by the penalty of a high mass so that the cuttlebone is required to combine high compressive strength with minimum weight.

The sealed chambers have a two-fold function. Firstly, they serve as individual compartments in which the relative amounts of gas and liquid can be regulated and hence the buoyancy of the animal controlled. Secondly, they provide a structure which combines a high porosity (93%) and low specific gravity (0.19) with the ability to resist external pressures greater than 1 MPa. Denton *et al.* [9] have reported that under hydrostatic pressure a whole cuttlebone withstood a pressure of 2.4 MPa (corresponding to a depth of 230 m) before imploding. The present studies indicate that when compressive failure does occur it is not catastrophic (as would be the case given a rigid but completely hollow organ) but is progressive (Fig. 10). Figs. 3 to 5 show the internal structure to consist of parallel layers separated and supported by pillars having an S-shaped cross-section. These contribute to compressive stability, for this cross-section minimizes any tendency for pillars to buckle by maximizing the second moment of area.

The dorsal shield provides a tough, strong cover for the lamellar matrix. It has a layered, non-porous structure with a very high organic content (30 to 40% by weight) and this is probably responsible for the toughness of the shield material. Currey [10] has shown that the toughness of bone is at an optimum when the organic content is about 34%. It is clear that the dorsal shield has an important mechanical role.

3.2. The role of the organic component

The ability of the organic component of the dorsal shield to undergo plastic deformation and hence to increase the toughness of the composite structure is unlikely to be the only role for the organic component of the cuttlebone. Indeed it has been widely proposed that the role of organic polymers in biological minerals is to control the growth and maintain the boundaries and shape of the structure (see for example [11, 12]). In the present study, it is shown that the removal of the inorganic phase leaves behind an organic relic of the structure (Fig. 7) and Fig. 9 shows how the inorganic structure is enveloped and bounded by a membrane-like organic material. The organic component thus appears to both initiate and limit the growth of the inorganic phase. The insoluble organic matrix may act as a template for the nucleation of the inorganic solid. This may be through groups on the polymer capable of interacting with ions in the solution phase or with nuclei of the solid phase that would be unstable unless so bound. In this respect, the importance of proteins containing gamma-carboxy-

glutamic acid (which binds Ca^{2+}) in calcium salt mineralization is now recognized [13]. Whilst insoluble polymers may initiate crystal growth, there is evidence that soluble polymeric components inhibit crystallization in the bulk [14] and may thus serve to restrict nucleation to the insoluble polymer matrix–an essential requirement if faithful reproduction of the template is to be attained. It has been observed [15] that in the crystallization of sodium chloride from solution, nucleation is inhibited by a dissolved polymer but is enhanced when the same polymer is insolubilized. Thus, it seems likely that the complex morphology of biomineral structures is determined by an insoluble organic template with nucleation restricted to the template by soluble components.

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